Task Group 7B: Cellular and Molecular Mechanisms of Biological Aging: The Roles of Nature, Nurture and Chance in the Maintenance of Human Healthspan

TASK GROUP DESCRIPTION

Background

The degree to which an individual organism maintains healthspan and lifespan is a function of complex interactions between genetic inheritance ("nature"), environment, including cultural inheritance (nurture) and stochastic events ("luck" or "chance"). This task group will focus upon the role of chance because it is so poorly understood and because it appears to be of major importance in the determination of individual variations in healthspan and lifespan *within* species. The major factor determining variations in healthspan and lifespan *between* species is genetic inheritance. Broader aspects of cellular and molecular mechanisms of biological aging will also be considered, given their importance for understanding the cellular and molecular basis of successful aging. The task force will consider the cellular and molecular basis for nature, nurture and chance in healthspan and life span determination.

On the basis of comparisons between identical and non-identical twins, geneticists have estimated that genes control no more than about a quarter of the inter-individual differences in lifespan (Herskind 1996). Twin studies of very old individuals, however, show substantially greater genetic contributions to Healthspan (McClearn 2004; Reed 2003). The environment clearly plays an important role in the length and the quality of life. Tobacco smoke, for example has the potential to impact upon multiple body systems in ways that appear to accelerate the rates at which those systems age (Bernhard 2007).

To document the role of chance events on aging, one must rigorously control both the genetic composition of an organism and its environment. This has been done to a remarkable degree in a species of nematodes, *Caenorhabditis elegans* (Vanfleteren 1998). The results confirm hundreds of previous studies with a wide range of species, especially those with inbred rodents housed under apparently identical but less well controlled environments. One observes wide variations in lifespan in all these studies. For the *C. elegans* experiments, the distributions of lifespan fit best with two parameter or three parameter logistic models and not with the classical Gompertz model nor the Weibull model.

Many mutations have been shown to substantially increase lifespan in *C. elegans*. It is of interest, however, that the ranges of the lifespan variations among such mutant strains overlap with those of wild type strains (Kirkwood 2002). Many of these long-lived mutant strains exhibit enhanced resistance to a variety of stressors, notably heat shock. It was therefore predicted that variable degrees of response to heat shock stress might form a basis, or a partial basis, for individual variations in longevity. An initial set of experiments demonstrated that is indeed the case, at least for a transgenic construct that includes the promoter of a small heat shock gene (Rea 2005). There was a very strong correlation between the response to a heat stress and longevity, with good responding worms living longer. Strikingly, this phenotype was not heritable. The progeny of a worm showing a strong heat stress reaction exhibited the broad distribution of lifespans shown by the starting

population. The heat stress reaction was therefore stochastic. The nature of the chance events that determine the reaction remains unknown. They could be related to the intrinsic instability of the transgene, making it important to repeat such experiments utilizing endogenous genes as reporters of the response to heat shock and other stressors. It could be due to epigenetic drifts in gene expression, perhaps involving random changes in gene promoters or in the state of chemical modifications to histone proteins that coat chromosomes. Such changes have indeed been observed in aging human identical twins (Fraga 2005). While those changes have been interpreted as being driven by the environment, one cannot at present rule out random variations unrelated to environmental influences.

Variations in gene expression in genetically identical organisms examined under environmentally identical conditions have also been attributable to intrinsic "noise" in fundamental molecular processes such as the transcription and translation of genes. Most such observations have been made using microorganisms (Elowitz 2002), but stochastic bursts of transcription have also been noted in mammalian cells (Raj 2006). Moreover, substantial variation in the levels at which genes are transcribed have shown to occur in mouse tissues, and that variation was shown to increase with age (Bahar 2006).

Chance events are also of major significance in the determination of diseases of aging. For the case of cancer, mutations have been shown to be of major importance. A likely key to malignancy, however, is the chance event of suffering a mutation in a gene which, when mutated, now greatly enhances the general frequency of mutation. Such genes are referred to as "mutator genes" (Bielas 2006). Chance events can make the difference between life and death of individuals with coronary artery atherosclerosis, as mortality often follows the rupture of an atherosclerotic plaque, an event that is likely to be due, in part, to a chance event (a "trigger") leading to the rupture (Falk 1992). Moreover, some genetic interventions that have been introduced into model organisms (nematodes, mice) increase mean but not maximum life span and appear to rectangularize the life span curve. A recent example is a mouse strain carrying extra copies of a tumor suppressor locus (Matheu 2007). As expected, these mice are remarkably cancer free. Of particular interest, though, their mean, but not maximum, life span was extended. Does this locus, and similar interventions, rectangularize the life span curve by reducing random events?

<u>Initial Challenges to Consider</u>

- What experiments might be designed in model organisms to probe the role of variations of endogenous gene expression at birth in the determinations of the remarkable stochastic variations in life span among genetically identical organisms?
- Which subset of endogenous genetic loci are major contributors to such stochastic variation?
- Are these variations in gene expression attributable to specific molecular events, such as chemical modifications to DNA CpG islands or histones?
- What are the molecular and biophysical mechanisms that lead to transcriptional bursts in gene expression?

- Do cells within an individual organism differ in their susceptibility to stochastic fluctuations in gene expression? For example, are post-mitotic neurons more or less susceptible than cells that are destined to die or cells that turn over?
- Are there species-specific differences in the degree to which stochastic fluctuations in gene expression occur (in similar cell types)?
- Has evolution shaped the above stochastic variations in gene expression, are they adaptive, and what are the selective pressures that led to such adaptations? How can one test the hypothesis that different degrees of stochastic variations in gene expression do in fact evolve and that they are adaptive?
- To what extent do early environmental influences in developing humans (fetal, neonatal, childhood, pubertal) determine patterns of gene expression and patterns of aging in human subjects?
- Do genetic interventions that increase mean but not maximum life span, and appear to rectangularize the life span curve, act by reducing random events? Can we learn about the cellular and molecular bases for stochastic variation by testing the hypothesis that some of these interventions act by this mechanism (reducing stochastic variation)?
- What are some candidate environmental agents and social influences responsible for such putative influences and how can their impacts upon the public health be measured?

Initial References

Bahar R, Hartmann CH, Rodriguez KA, Denny AD, Busuttil RA, Dolle ME, Calder RB, Chisholm GB, Pollock BH, Klein CA, Vijg J. Increased cell-to-cell variation in gene expression in ageing mouse heart. Nature 2006;441:1011-1014.

Bernhard D, Moser C, Backovic A, Wick G. Cigarette smoke - an aging accelerator? *Exp* Gerontol 2007 Mar;42(3):160-5. Epub 2006 Nov 3.

Bielas JH, Loeb KR, Rubin BP, True LD, Loeb LA. Human cancers express a mutator phenotype. Proc Natl Acad Sci *USA* 2006;103:18238-18242.

Elowitz MB, Levine AJ, Siggia ED, Swain PS. Stochastic gene expression in a single cell. Science 2002;297:1183-1186.

Falk E. Why do plaques rupture? Circulation 1992;86:III30-III42.

Fraga MF, Ballestar E, Paz MF, Ropero S, Setien F, Ballestar ML, Heine-Suner D, Cigudosa JC, Urioste M, Benitez J, Boix-Chornet, M, Sanchez-Aguilera A, Ling C, Carlsson E, Poulsen P, Vaag A, Stephan Z, Spector TD, Wu YZ, Plass C, Esteller M. Epigenetic differences arise during the lifetime of monozygotic twins. Proc Natl Acad Sci *USA* 2005;102:10604-10609.

Herskind AM, McGue M, Holm NV, Sorensen TI, Harvald B, Vaupel JW. The heritability of human longevity: a population-based study of 2872 Danish twin pairs born 1870-1900. Hum Genet 1996;97: 319-323.

Kirkwood TB, Finch CE. Ageing: the old worm turns more slowly. Nature 2002;419: 794 795.

Matheu A, Maraver A, Klatt P, Flores I, Garcia-Cao I, Borras C, Flores JM, Viña J, Blasco MA, Serrano M. Delayed ageing through damage protection by the Arf/p53 pathway. Nature 2007; 448:375-379.

McClearn G E, Johansson B, Berg S, Pedersen NL, Ahern F, Petrill S A, Plomin, R.

Substantial genetic influence on cognitive abilities in twins 80 or more years old. Science 1997;276:1560-1563.
Raj A, Peskin CS, Tranchina D, Vargas DY, Tyagi S. Stochastic mRNA synthesis in mammalian cells. PLoS Biol 2006;4:e309.
Rea SL, Wu D, Cypser JR, Vaupel JW, Johnson TE. A stress-sensitive reporter predicts longevity in isogenic populations of caenorhabditis elegans. Nat Genet 2005;37:894-898.
Reed T, Dick DM. Heritability and validity of healthy physical aging (wellness) in elderly male twins. Twin Res 2003;6:227-234.
Vanfleteren JR, De VA, Braeckman BP. Two-parameter logistic and Weibull equations provide better fits to survival data from isogenic populations of Caenorhabditis elegans in axenic culture than does the Gompertz model. J Gerontol A Biol Sci Med Sci. 1998;53:B393-B403.

Task Group Members:

- Linda Miller, Nature
- Christine Grant, North Carolina State University
- Su-Ju Lin, University of California Davis
- Jerry Shay, University of Texas Southwestern Medical Center
- Heinz-Ulrich G. Weier, E.O. Lawrence Berkeley National Laboratory
- Eric Topol, Scripps Research Institute
- Suresh Arya, National Cancer Institute
- Richard Miller, University of Michigan
- Michael Torry, Steadman-Hawkins Research/Foundation
- Chris Patil, Lawrence Berkeley National Laboratory
- Santa Jeremy Ono, Emory University
- Iris Tse, Boston University

TASK GROUP SUMMARY

Summary written by Iris Tse, Graduate Student, Boston University

> *I have cataracts, lose uphill races, get really sick when I catch the flu, girls no longer whistle when I pass by, my joints ache, and if you looked closely you'd see preclinical signs of the cancer that will kill me.*
>
> *How Old am I?*

All species of animals do not age at the same rate. Humans, inevitably, will have a longer lifespan than horses, which in turn, will have a longer lifespan than most rodents. However, there is an unexpected synchronicity in the way age-related diseases appear across different species. At certain points in life, all of these animals will get vision degeneration, cancer, diabetes, CNS degeneration and organ failures. Even more surprising is that most of these age-related diseases will appear in the similar sequence, regardless of lifespan and size of the animals. It appears as if some yet unknown species- or breed-specific factors are tying

together these functional changes. Therefore the group found this to be an area worth pursuing.

The validity of the stochastic model

Before settling on this topic, the multidisciplinary group first debated in earnest the validity of the stochastic model as a formative criterion for healthspan and lifespan. The stochastic model was initially assigned to the group by the conference organizers as a springboard for discussion. Previous research has found that there is a wide range of lifespan within genetically identical cohort. Therefore, there must be some ephemeral non-genetic factor that can influence the aging trajectory and lifespan. Aging is not a 100 per cent deterministic issue and undefined gaps in current knowledge still needs to be examined and studies.

However, some members of the group felt that even though there are undefined gaps in current knowledge, it would be premature to attribute the entirety of this unknown area to the stochastic model. The stochastic model, while important, is not the complete answer.

After an hour of vigorous discussion and scrutiny, the group leader, Jerry Shay, a professor of cell biology at UT Southwestern Medical Center, distilled the group's thinking.

"To think that aging is stochastic, and therefore not within our control, greatly diminishes the impact and window of ability to understand, change and manipulate the process of aging," said Shay.

Important topics surrounding lifespan and healthspan

Once the group discarded the stochastic model, the group members were free to explore other facets of aging. Linda Miller, the US Executive Editor of *Nature* and the Nature Journals, sparked the next round of discussion by pointing out neuro-degeneration as an area worth exploring. Topics suggested by other members of the group included: cell regenerative capacity, cellular replication and control, broad spectrum genomic analysis of aging, the frequency of cancerous tumor seen in post-reproductive period and biomaterials used in current research.

However, the topic that seized the group's attention was the synchronicity of age-associated decline. Richard Miller, a professor of pathology and geriatrics at the University of Michigan pointed out that many organs and cells fail at more or less the same time within a species. The sequence of illnesses are often synchronized across various species of animals, albeit at a different rate and adjusted to the expected lifespan of specific species. These co-morbid events, such as the onset of diabetes or cataracts, are not necessarily terminal illnesses and may not directly affect lifespan. However, they do have a huge effect on healthspan. If we can understand why humans get cataracts at 60-years-old and how mice get cataracts at 2-years-old, we might be able to delay the onset of these symptoms, prolong healthspan and understand aging a little better.

Miller insisted that it will be useful to find themes, or common families, of underlying cellular or molecular factors that time aging sequentially. The initial goal will be to develop a new set of hypothesis for future experiments.

Experimental approach

Miller already had a rough idea of the experimental approach and the group spent the remaining day and half crystallizing those points. An important first step is to generate a list of late life dysfunctions plausibly related to the timing of aging. These age-related dysfunctions can be diseases, such as cancer, or symptoms, such as cataracts or cognitive failure, that affect many animals.

Experts knowledgeable in aging or pathogenesis, or both, will be solicited to provide these hypotheses. They also will be called upon to brainstorm a body of known factors, such as proteins, macromolecules, enzymatic activities and genetic expressions that might influence the aforementioned list of diseases. By understanding the synchronicity of these factors, perhaps we will understand more about the synchronicity of aging.

Since the group hoped to get the experiment started as quickly as possible, an important criterion to these hypotheses is that these factors must be easy and practical to measure using established methodology and reagents. A protocol using existing technology and knowledge of animals or research process will be favored over developing a novel process from scratch. For examples, we can look at cellular events such as ubiquitiation (or other regulatory factors or non-coding sequences), that doesn't require species-specific antibodies.

"Basically, you would want to be able use preexisting reagents or kits straight from a Sigma catalogue. Otherwise, you'd get mired over things like the trial and error of a new experimental design," said Chris Patil, a postdoctoral scholar of life sciences from Lawrence Berkeley National Laboratory.

The next step will be to select the proper animal models for interspecies comparison. The experiments will use healthy young adults with no age-related diseases because the selective factors that mold maturation is not the same as aging. These animals will be evaluated to uncover patterns of protein expressions, or possibly some other cellular events, that determine the rate of aging of that particular species.

Four types of animals were chosen for this large-scale study:

Primates – for their similarity to humans
Bats – in most animals, lifespan is directly correlated to body weight. However, bats have a long lifespan for their small body size, therefore allowing researchers to observe trends that are actually related to lifespan and not weight.

Rodents – lifespan varies drastically across different species of rodents, ranging from 2 years for mice to 30 years for naked mole rats. The variety allows experimenters to adjust for confounders.

Birds – the non-mammalian outgroup to anchor the phylogenetic tree during statistical analysis.

Additional funding will be necessary to trap and collect these animals, around ten species for each clade of animals. Animals from the wild will be ideal, since they provide a more realistic snapshot of the aging process. But, the group is also open to using captive animals, such as those from zoos, since this will hasten the process and likely to reduce cost.

This broad-spectrum analysis is meant to generate useful hypotheses. It's not meant to be a hypothesis-testing procedure. The end product from these experiments will produce information necessary to created mechanistic hypothesis for further testing. It is important to provoke further questions and examinations. Interspecies contrast will be the first step. Once something comes up as a hit, then the next step will be intraspecies comparison.

"Instead of approaching diseases one at a time, which will take up too much time and it's a narrow-minded way to do things," said Miller.

"This umbrella approach is a better way to probe the age/diseases nexus by exploiting the power of comparison."